

1 We claim:

- 2 1. A lentiviral vector comprising the following elements: a nucleic acid whose  
3 sequence includes (i) a functional packaging signal; (ii) a multiple cloning site  
4 (MCS); and (iii) at least one additional element selected from the group consisting  
5 of: a second MCS, a second MCS into which a heterologous nucleic acid is inserted,  
6 an HIV FLAP element, an expression-enhancing posttranscriptional regulatory  
7 element, a target site for a site-specific recombinase, and a self-inactivating (SIN)  
8 LTR, wherein the lentiviral vector is a lentiviral transfer plasmid or an infectious  
9 lentiviral particle.
- 10 2. The lentiviral vector of claim 1, wherein the vector comprises at least two elements  
11 selected from the group consisting of: a second MCS, a second MCS into which a  
12 heterologous nucleic acid is inserted, an HIV FLAP element, an expression-  
13 enhancing posttranscriptional regulatory element, a target site for a site-specific  
14 recombinase, and a self-inactivating (SIN) LTR.
- 15 3. The lentiviral vector of claim 1, wherein the vector comprises at least three elements  
16 selected from the group consisting of: a second MCS, a second MCS into which a  
17 heterologous nucleic acid is inserted, an HIV FLAP element, an expression-  
18 enhancing posttranscriptional regulatory element, a target site for a site-specific  
19 recombinase, and a self-inactivating (SIN) LTR.
- 20 4. The lentiviral vector of claim 1, wherein the vector comprises at least four elements  
21 selected from the group consisting of: a second MCS, a second MCS into which a  
22 heterologous nucleic acid is inserted, an HIV FLAP element, an expression-  
23 enhancing posttranscriptional regulatory element, a target site for a site-specific  
24 recombinase, and a self-inactivating (SIN) LTR.
- 25 5. The lentiviral vector of claim 1, wherein the vector comprises a second MCS, an  
26 HIV FLAP element, an expression-enhancing posttranscriptional regulatory element,  
27 a target site for a site-specific recombinase, and a self-inactivating (SIN) LTR.
- 28 6. The lentiviral vector of claim 1, wherein the vector comprises a second MCS into  
29 which a heterologous nucleic acid is inserted, an HIV FLAP element, an expression-

- 1 enhancing posttranscriptional regulatory element, a target site for a site-specific  
2 recombinase, and a self-inactivating (SIN) LTR.
- 3 7. The lentiviral vector of claim 1, wherein the additional element is a second MCS.
- 4 8. The lentiviral vector of claim 1, wherein the additional element is a second MCS into  
5 which a heterologous nucleic acid is inserted.
- 6 9. The lentiviral vector of claim 1, wherein the vector has unique restriction sites for at  
7 least 4 enzymes selected from the group consisting of NotI, ApaI, XhoI, XbaI, HpaI,  
8 NheI, PacI, NsiI, SphI, Sma/Xma, AccI, BamHI, and SphI.
- 9 10. The lentiviral vector of claim 1, wherein the vector has unique restriction sites for at  
10 least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, or  
11 at least 13 enzymes selected from the group consisting of NotI, ApaI, XhoI, XbaI,  
12 HpaI, NheI, PacI, NsiI, SphI, Sma/Xma, AccI, BamHI, and SphI.
- 13 11. The lentiviral vector of claim 1, wherein the additional element is an HIV FLAP  
14 element.
- 15 12. The lentiviral vector of claim 1, wherein the additional element is an expression-  
16 enhancing posttranscriptional regulatory element.
- 17 13. The lentiviral vector of claim 12, wherein the expression-enhancing  
18 posttranscriptional regulatory element is a WRE.
- 19 14. The lentiviral vector of claim 1, wherein the additional element is a target site for a  
20 site-specific recombinase.
- 21 15. The lentiviral vector of claim 14, wherein the site is a loxP site.
- 22 16. The lentiviral vector of claim 1, wherein the lentiviral vector is a lentiviral transfer  
23 plasmid.
- 24 17. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less  
25 than 10 kB.

- 1 18. The lentiviral transfer plasmid of claim 16, wherein the plamid has a size of less than  
2 9 kB.
- 3 19. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less  
4 than 8 kB.
- 5 20. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of less  
6 than 7 kB.
- 7 21. The lentiviral transfer plasmid of claim 16, wherein the plasmid has a size of  
8 approximately 6 kB.
- 9 22. The lentiviral vector of claim 1, wherein the lentiviral vector is an infectious  
10 lentiviral particle.
- 11 23. The lentiviral vector of claim 1, further comprising: a heterologous promoter or  
12 promoter-enhancer.
- 13 24. The lentiviral vector of claim 23, wherein the heterologous promoter or promoter-  
14 enhancer is selected from the group consisting of: the CMV promoter, the CMV  
15 promoter-enhancer, and the ubiquitin C promoter.
- 16 25. The lentiviral vector of claim 24, wherein the heterologous promoter is an inducible  
17 promoter.
- 18 26. The lentiviral vector of claim 24, wherein the heterologous promoter is a cell type  
19 specific or tissue specific promoter.
- 20 27. The lentiviral vector of claim 23, wherein the heterologous promoter is an RNA  
21 polymerase promoter.
- 22 28. The lentiviral vector of claim 27, wherein the RNA polymerase promoter is an RNA  
23 polymerase III promoter.
- 24 29. The lentiviral vector of claim 28, wherein the RNA polymerase III promoter is a U6  
25 promoter.

- 1 30. The lentiviral vector of claim 28, wherein the RNA polymerase III promoter is an H1  
2 promoter.
- 3 31. The lentiviral vector of claim 27, wherein the RNA polymerase promoter is an RNA  
4 polymerase II promoter.
- 5 32. The lentiviral vector of claim 23, further comprising a second heterologous promoter  
6 or promoter-enhancer.
- 7 33. The lentiviral vector of claim 1, further comprising a heterologous nucleic acid  
8 encoding a selectable marker operably linked to a promoter.
- 9 34. The lentiviral vector of claim 1, further comprising a heterologous nucleic acid  
10 encoding a reporter molecule operably linked to a promoter.
- 11 35. The lentiviral vector of claim 34, wherein the reporter molecule is selected from the  
12 group consisting of: GFP, EGFP, dsRed, dsRed2, cyan fluorescent protein, yellow  
13 fluorescent protein, blue fluorescent protein, dsRed, dsRed2, luciferase, and  
14 aequorin.
- 15 36. The lentiviral vector of claim 34, further comprising an RNA polymerase promoter.
- 16 37. The lentiviral vector of claim 36, wherein the RNA polymerase promoter is an RNA  
17 polymerase III promoter.
- 18 38. The lentiviral vector of claim 1, wherein the lentiviral vector is a transfer plasmid,  
19 further comprising a genetic element sufficient for stable maintenance of the transfer  
20 plasmid as an episome within mammalian cells.
- 21 39. A lentiviral vector comprising an RNA polymerase III promoter.
- 22 40. The lentiviral vector of claim 39, wherein the RNA polymerase III promoter is a U6  
23 promoter.
- 24 41. The lentiviral vector of claim 39, wherein the RNA polymerase III promoter is an H1  
25 promoter.

- 1 42. The lentiviral vector of claim 39, further comprising a heterologous nucleic acid  
2 encoding a reporter molecule.
- 3 43. A lentiviral vector having a sequence as set forth in SEQ ID NO: 2, SEQ ID NO: 3,  
4 SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or  
5 SEQ ID NO: 9.
- 6 44. A collection of at least two of the lentiviral vectors of claim 43.
- 7 45. The collection of claim 44, wherein the collection includes a vector comprising a  
8 first heterologous promoter element and a vector comprising a second heterologous  
9 promoter element different from the first promoter element.
- 10 46. The collection of claim 44, wherein the collection includes a vector comprising a  
11 first heterologous reporter gene and a vector comprising a second reporter gene  
12 different from the first reporter gene.
- 13 47. A lentiviral vector having a sequence that differs by not more than 100 nucleotides  
14 from the sequence set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ  
15 ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.
- 16 48. A collection of at least two of the lentiviral vectors of claim 47.
- 17 49. The collection of claim 47, wherein the collection includes a vector comprising a  
18 first heterologous promoter element and a vector comprising a second heterologous  
19 promoter element different from the first promoter element.
- 20 50. The collection of claim 47, wherein the collection includes a vector comprising a  
21 first heterologous reporter gene and a vector comprising a second reporter gene  
22 different from the first reporter gene.
- 23 51. A lentiviral vector having a sequence that differs by not more than X nucleotides  
24 from the sequence set forth in SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ  
25 ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9, where  
26 X represents any number between 1 and 99, inclusive.
- 27 52. A collection of at least two of the lentiviral vectors of claim 51.

- 1     53.     A collection of at least two of the lentiviral vectors of claim 51.
- 2     54.     The collection of claim 53, wherein the collection includes a vector comprising a  
3           first heterologous promoter element and a vector comprising a second heterologous  
4           promoter element different from the first promoter element.
- 5     55.     The collection of claim 53, wherein the collection includes a vector comprising a  
6           first heterologous reporter gene and a vector comprising a second reporter gene  
7           different from the first reporter gene.
- 8     56.     A three-plasmid lentiviral expression system comprising:  
9           (a) a first plasmid whose sequence comprises a nucleic acid sequence of at  
10          least part of a lentiviral genome, wherein the plasmid (i) contains at least one defect  
11          in at least one gene encoding a lentiviral structural protein, and (ii) lacks a functional  
12          packaging signal;  
13          (b) a second plasmid whose sequence comprises a nucleic acid sequence of a  
14          virus, wherein the plasmid (i) expresses a viral envelope protein, and (ii) lacks a  
15          functional packaging signal; and  
16          (c) a third plasmid whose nucleic acid sequence includes (i) a functional  
17          packaging signal; (ii) a multiple cloning site (MCS); and (iii) at least one additional  
18          element selected from the group consisting of: a second MCS, a second MCS into  
19          which a heterologous nucleic acid is inserted; an HIV FLAP element, an expression-  
20          enhancing posttranscriptional regulatory element, a target site for a site-specific  
21          recombinase, and a self-inactivating (SIN) LTR.
- 22     57.     A four plasmid lentiviral expression system comprising the three plasmid lentiviral  
23           expression system of claim 56, further comprising a fourth plasmid comprising a  
24           nucleic acid segment that encodes Rev, operably linked to a promoter.
- 25     58.     A cell comprising the lentiviral vector of claim 1.
- 26     59.     The cell of claim 58, wherein the cell comprises a nucleic acid or nucleic acids  
27           having sequences encoding Gag, Pol, and Env proteins.
- 28     60.     A cell comprising a provirus derived from the lentiviral vector of claim 1.

- 1 61. A transgenic animal, at least some of whose cells contain the lentiviral vector of  
2 claim 1.
- 3 62. A transgenic animal, at least some of whose cells contain a provirus derived from the  
4 lentiviral vector of claim 1.
- 5 63. A method of creating a producer cell line comprising introducing the lentiviral vector  
6 of claim 1 into a host cell, wherein the lentiviral vector is a transfer plasmid; and  
7 introducing a packaging plasmid and an envelope plasmid into the host cell.
- 8 64. A method of producing lentiviral particles comprising  
9 (i) introducing the lentiviral vector of claim 1 into a helper cell, wherein the  
10 lentiviral vector is a transfer plasmid comprising a genetic element sufficient for  
11 stable maintenance of the plasmid as an episome in mammalian cells, into a helper  
12 cell that expresses proteins required for production of infectious lentiviral particles;  
13 and  
14 (ii) culturing the cell for a period sufficient to allow production of lentiviral  
15 particles.
- 16 65. A method of producing lentiviral particles comprising  
17 (i) introducing the lentiviral vector of claim 1, which lentiviral vector is a  
18 lentiviral transfer plasmid comprising a genetic element sufficient for stable  
19 maintenance of the transfer plasmid as an episome in mammalian cells, into a helper  
20 cell that expresses a protein required for production of lentiviral particles, wherein  
21 expression of the protein is under control of an inducible promoter;  
22 (ii) inducing expression of the protein required for production of lentiviral  
23 particles; and  
24 (iii) culturing the cell for a period sufficient to allow production of lentiviral  
25 particles.
- 26 66. A method of expressing a heterologous nucleic acid in a target cell comprising  
27 introducing a lentiviral vector of claim 1 into the target cell, wherein the  
28 lentiviral vector comprises a heterologous nucleic acid operably linked to a  
29 promoter; and  
30 expressing the heterologous nucleic acid therein.

- 1 67. A method for achieving controlled expression of a heterologous nucleic acid in a cell  
2 comprising steps of:  
3 (i) providing a modified lentiviral vector comprising a heterologous nucleic  
4 acid inserted between sites for a recombinase;  
5 (ii) introducing the modified lentiviral vector or a portion thereof including at  
6 least the sites for the recombinase and the region between the sites into the cell and;  
7 (iii) subsequently inducing expression of the recombinase within the cell,  
8 thereby preventing expression of the heterologous nucleic acid within the cell.
- 9 68. The method of claim 67, wherein the providing step comprises inserting the  
10 heterologous nucleic acid into a lentiviral vector between sites for a recombinase,  
11 thereby producing a modified lentiviral vector.
- 12 69. The method of claim 67, wherein the cell is a mammalian cell.
- 13 70. A method for expressing a transcript in a mammal in a cell type or tissue-specific  
14 manner comprising:  
15 (i) delivering a lentiviral vector to cells of the mammal, wherein the lentiviral  
16 vector comprises a heterologous nucleic acid operably linked to a promoter so that  
17 transcription from the promoter results in synthesis of the transcript, and wherein the  
18 heterologous nucleic acid is located between sites for a site-specific recombinase;  
19 and  
20 (ii) inducing expression of the site-specific recombinase in a subset of the  
21 cells of the mammal, thereby preventing synthesis of the transcript within those cells.
- 22 71. The method of claim 67 or claim 70, wherein the step of inducing the site-specific  
23 recombinase comprises introducing a vector encoding the site-specific recombinase  
24 into the cell.
- 25 72. The method of claim 67 or claim 70, wherein expression of the site-specific  
26 recombinase is under control of a cell type specific or tissue specific promoter.
- 27 73. The method of claim 67 or claim 70, wherein the sites are loxP sites and the site-  
28 specific recombinase is loxP.



- 1     74.     A lentiviral vector whose presence within a cell results in transcription of one or  
2             more ribonucleic acids (RNAs) that self-hybridize or hybridize to each other to form  
3             a short hairpin RNA or short interfering RNA that inhibits expression of at least one  
4             target transcript in the cell.
- 5     75.     The lentiviral vector of claim 74, wherein the vector provides a template for  
6             synthesis of an RNA that self-hybridizes to form an shRNA that is targeted to the  
7             transcript.
- 8     76.     The lentiviral vector of claim 75, wherein the shRNA comprises a loop having a  
9             sequence set forth in SEQ ID NO: 10.
- 10    77.     The lentiviral vector of claim 74, wherein the vector provides a template for  
11             synthesis of complementary RNAs that hybridize with each other to form an siRNA  
12             that is targeted to the transcript.
- 13    78.     The lentiviral vector of claim 74, wherein the vector comprises a nucleic acid  
14             segment operably linked to a promoter, so that transcription from the promoter  
15             results in synthesis of one or more RNAs that self-hybridize or hybridize with each  
16             other to form an shRNA or siRNA targeted to the transcript.
- 17    79.     The composition of claim 74, wherein the lentiviral vector is a lentiviral transfer  
18             plasmid.
- 19    80.     The composition of claim 74, wherein the lentiviral vector is an infectious lentiviral  
20             particle.
- 21    81.     The lentiviral vector of claim 74, wherein:  
22                 the shRNA or siRNA comprises a base-paired region approximately 19  
23             nucleotides long.
- 24    82.     The lentiviral vector of claim 74, wherein:  
25                 the shRNA or siRNA comprises a base-paired region and at least one single-  
26             stranded overhang.
- 27    83.     The lentiviral vector of claim 74 wherein:

1                   the siRNA or shRNA comprises a 3' overhang consisting of at least two  
2                   pyrimidines.

3   84.    The lentiviral vector of claim 83, wherein the 3' overhang is UU.

4   85.    The lentiviral vector of claim 74, wherein:

5                   the shRNA or siRNA comprises a region that is precisely complementary  
6                   with a region of the target transcript.

7   86.    The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level  
8                   sufficient to reduce the level of the target transcript or its encoded protein by at least  
9                   about 2 fold.

10   87.   The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level  
11                   sufficient to reduce the level of the target transcript or its encoded protein by at least  
12                   about 5 fold.

13   88.   The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level  
14                   sufficient to reduce the level of the target transcript or its encoded protein by at least  
15                   about 10 fold.

16   89.   The lentiviral vector of claim 74, wherein the siRNA or shRNA is present at a level  
17                   sufficient to reduce the level of the target transcript or its encoded protein by at least  
18                   about 25 fold.

19   90.    The lentiviral vector of claim 74, wherein the lentiviral vector comprises:

20                   (i) a functional packaging signal;

21                   (ii) a multiple cloning site (MCS); and

22                   (iii) at least one additional element selected from the group consisting of: a  
23                   second MCS, a second MCS into which a heterologous promoter or promoter-  
24                   enhancer is inserted, an HIV FLAP element, an expression-enhancing  
25                   posttranscriptional regulatory element, a target site for a site-specific recombinase,  
26                   and a self-inactivating (SIN) LTR.

27   91.    A composition comprising:

28                   the lentiviral vector of claim 74; and

- 1                   a delivery agent that enhances delivery of the vector to cells.
- 2   92.   A pharmaceutical composition comprising:
- 3                   the lentiviral vector of claim 74; and
- 4                   a pharmaceutically acceptable carrier.
- 5   93.   A three plasmid lentiviral expression system comprising (i) a lentiviral transfer
- 6                   plasmid comprising a heterologous nucleic acid operably linked to a promoter, so
- 7                   that transcription of the heterologous nucleic acid produces one or more RNAs that
- 8                   self-hybridize or hybridize with each other to form an shRNA or siRNA targeted to a
- 9                   target transcript; (ii) a packaging plasmid; and (iii) an Env-coding plasmid.
- 10   94.   A four plasmid lentiviral expression system comprising the three plasmid lentiviral
- 11                   expression system of claim 93, further comprising a fourth plasmid comprising a
- 12                   nucleic acid segment that encodes Rev, operably linked to a promoter.
- 13   95.   A method of inhibiting or reducing the expression of a target transcript in a cell
- 14                   comprising delivering the lentiviral vector of claim 74 to the cell.
- 15   96.   The method of claim 95, wherein the lentiviral vector comprises:
- 16                   (i) a functional packaging signal;
- 17                   (ii) a multiple cloning site (MCS); and
- 18                   (iii) at least one additional element selected from the group consisting of: a
- 19                   second MCS, a second MCS into which a heterologous promoter or promoter-
- 20                   enhancer is inserted, an HIV FLAP element, an expression-enhancing
- 21                   posttranscriptional regulatory element, a target site for a site-specific recombinase,
- 22                   and a self-inactivating (SIN) LTR .
- 23   97.   The method of claim 95, wherein the cell is a mammalian cell.
- 24   98.   The method of claim 95, wherein the cell is a primary cell.
- 25   99.   The method of claim 95, wherein the primary cell is a T cell.
- 26   100.   The method of claim 95, wherein the cell is a non-dividing cell.
- 27   101.   The method of claim 95, wherein the cell is an embryonic stem cell.

- 1    102.    The method of claim 95, wherein the cell is a single-cell embryo.
- 2    103.    The method of claim 95, wherein the lentiviral vector is a lentiviral transfer plasmid.
- 3    104.    The method of claim 95, wherein the lentiviral vector is an infectious lentiviral  
4            particle.
- 5    105.    The method of claim 95, wherein the ribonucleic acid comprises complementary  
6            regions that self-hybridize to form a short hairpin RNA targeted to the transcript.
- 7    106.    A method of reversibly inhibiting or reducing expression of a target transcript in a  
8            cell comprising steps of:
- 9            (i) delivering a lentiviral vector to the cell, wherein presence of the lentiviral  
10            vector within the cell results in synthesis of one or more RNAs that self-hybridize or  
11            hybridize with each other to form an shRNA or siRNA that inhibits expression of the  
12            target transcript, wherein the lentiviral vector comprises a nucleic acid segment  
13            located between sites for a site-specific recombinase, which nucleic acid segment  
14            provides a template for transcription of the one or more RNAs; and (ii) inducing  
15            expression of the site-specific recombinase within the cell, thereby preventing  
16            synthesis of at least one of the RNAs.
- 17   107.    The method of claim 105, wherein the cell is a mammalian cell.
- 18   108.    The method of claim 105, wherein the recombinase is Cre and the sites are loxP  
19            sites.
- 20   109.    The method of claim 105, wherein the lentiviral vector is a lentiviral transfer  
21            plasmid.
- 22   110.    The method of claim 105, wherein the lentiviral vector is a lentiviral particle.
- 23   111.    The method of claim 105, wherein the lentiviral vector provides a template for  
24            synthesis of an RNA comprising complementary portions that hybridize to form an  
25            shRNA.
- 26   112.    A method for reversibly inhibiting or reducing expression of a transcript in a  
27            mammal in a cell type or tissue-specific manner comprising:

1 (i) delivering to the mammal a lentiviral vector whose presence within a cell  
2 results in synthesis of one or more RNAs that self-hybridize or hybridize with each  
3 other to form an shRNA or siRNA that inhibits expression of the target transcript,  
4 wherein the lentiviral vector comprises a nucleic acid segment located between sites  
5 for a site-specific recombinase, which nucleic acid segment provides a template for  
6 transcription of the RNA; and

7 (ii) inducing expression of the site-specific recombinase in a subset of the  
8 cells of the mammal, thereby preventing synthesis of at least one of the RNAs within  
9 the subset of cells.

10 113. The method of claim 112, wherein the recombinase is Cre and the sites are loxP  
11 sites.

12 114. The method of claim 112, wherein the lentiviral vector is a lentiviral transfer  
13 plasmid.

14 115. The method of claim 112, wherein the lentiviral vector is a lentiviral particle.

15 116. The method of claim 112, wherein the lentiviral vector provides a template for  
16 synthesis of an RNA comprising complementary portions that hybridize to form an  
17 shRNA.

18 117. A method of treating or preventing infection by an infectious agent, the method  
19 comprising steps of:  
20 administering to a subject prior to, simultaneously with, or after exposure of  
21 the subject to the infectious agent, a composition comprising an effective amount of  
22 a lentiviral vector, wherein presence of the lentiviral vector in a cell results in  
23 synthesis of one or more RNAs that self-hybridize or hybridize with each other to  
24 form an shRNA or siRNA that is targeted to a transcript produced during infection  
25 by the infectious agent, which transcript is characterized in that reduction in levels of  
26 the transcript delays, prevents, or inhibits one or more aspects of infection by or  
27 replication of the infectious agent.

- 1    118.    The method of claim 117, wherein the lentiviral vector provides a template for  
2            synthesis of an RNA that comprises complementary portions that hybridize to form  
3            an shRNA.
- 4    119.    A method of treating or preventing a disease or clinical condition, the method  
5            comprising:  
6                 removing a population of cells from a subject at risk of or suffering from  
7            disease or clinical condition;  
8                 engineering or manipulating the cells to contain an effective amount of an  
9            siRNA or shRNA targeted to a transcript, which transcript is characterized in that its  
10          degradation delays, prevents, or inhibits one or more aspects of the disease or clinical  
11          condition; and  
12          returning at least a portion of the cells to the subject.
- 13   120.    The method of claim 119 wherein:  
14            the engineering or manipulating step comprises introducing a lentiviral vector  
15            into the cells, wherein presence of the lentiviral vector in a cell results in synthesis of  
16            one or more RNAs that self-hybridize or hybridize with each other to form an  
17            shRNA or siRNA targeted to the transcript.
- 18   121.    The method of claim 119, wherein:  
19            the cells comprise stem cells.
- 20   122.    The method of claim 121, wherein:  
21            the stem cells are peripheral blood stem cells.
- 22   123.    The method of claim 119, further comprising:  
23            expanding at least a portion of the cells in culture.
- 24   124.    A kit comprising (a) a lentiviral transfer plasmid comprising a nucleic acid sequence  
25            including (i) a functional packaging signal; (ii) a multiple cloning site (MCS) into  
26            which a heterologous gene may be inserted; and (iii) at least one additional element  
27            selected from the group consisting of: a second MCS, an HIV FLAP element, a  
28            heterologous promoter, a heterologous enhancer, an expression-enhancing  
29            posttranscriptional regulatory element, a target site for a site-specific recombinase,

1 and a self-inactivating (SIN) LTR; and one or more of the following items: (b) a  
2 packaging mix comprising one or more plasmids that collectively provide nucleic  
3 acid sequences coding for retroviral or lentiviral Gag and Pol proteins and an  
4 envelope protein; (c) cells (e.g., a cell line) that are permissive for production of  
5 lentiviral particles such as 293T cells; (d) packaging cells, e.g., a cell line that is  
6 permissive for production of lentiviral particles and provides the proteins Gag, Pol,  
7 Env, and, optionally, Rev; (e) cells suitable for use in titering lentiviral particles; a  
8 transfection-enhancing agent such as Lipofectamine; (f) a selection agent such as an  
9 antibiotic, preferably corresponding to an antibiotic resistance gene in the lentiviral  
10 transfer plasmid; (g) instructions for use; (h) a positive control plasmid.

11